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B. T. Galloway, *Chief of Bureau.*

THE DETERMINATION OF THE DETERIORATION OF MAIZE, WITH INCIDENTAL REFERENCE TO PELLAGRA.

BY

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., August 20, 1910.

Sir: I have the honor to transmit herewith and to recommend for publication as Bulletin No. 199 of the series of this Bureau the accompanying manuscript entitled "The Determination of the Deterioration of Maize, with Incidental Reference to Pellagra," by Mr. Otis F. Black and Dr. Carl L. Alsberg, Chemical Biologists, which has been submitted for publication by Dr. Rodney H. True, Physiologist in Charge of Drug-Plant, Poisonous-Plant, Physiological, and Fermentation Investigations, of the Bureau of Plant Industry.

As a necessary preliminary step in the investigation of the alleged relation between spoiled corn and pellagra the authors of this paper have made a critical study of the methods of detecting products of deterioration in corn and corn meal. The recent recognition of pellagra in the United States has emphasized the fact that there is a lack of such information in a form available for English readers and has brought about a considerable demand for it. The accompanying paper deals critically with the value of methods employed in foreign countries and contains experimental data bearing upon their application to conditions in this country. The work constitutes a first step in the study of the constituents present in corn and the possible production of toxic substances by deterioration.

Respectfully,

G. H. Powell,
Acting Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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THE DETERMINATION OF THE DETERIORATION OF MAIZE, WITH INCIDENTAL REFERENCE TO PELLAGRA.

INTRODUCTION.

The recent recognition of cases of pellagra in this country, principally in the Southern States, and the supposed connection of the disease with the consumption of unsound corn[a] have called attention to the lack of methods by which to test the fitness of corn and corn meal for human consumption. There is every reason to believe that sound corn is a most wholesome food. Whether corn that has been heated, fermented, or molded is equally safe is another question. That it is unsafe and the cause of pellagra is so firmly believed in Italy and the Austrian Province of the Tyrol that the Governments of these countries have enacted stringent laws regulating the quality of corn and corn meal which may be sold or imported.[b] The possibility that spoiled corn may possess poisonous qualities seems to have passed unnoticed in this country.

Indeed, it is found that with the exception of such work as that of Osborne[c] and others upon the proteins, little work has been done

[a] The term "corn" is a general one, usually applied to the chief cereal crop of a country. It is therefore not necessarily applied everywhere to the same cereal. Thus, in England, it is applied to wheat, and in the United States to maize (Indian corn). The terms Indian corn, maize, and corn will be used interchangeably in this paper.

[b] Bollettino Ufficiale del Ministero d'Agricoltura, Industria, e Commercio, Rome, new series, vol. 4, no. 4, October, 1902, pp. 663–666.

Gesetz und Verordnungsblatt für die gefürstete Grafschaft Tyrol und das Land Vorarlberg, 1904, no. 12, p. 57.

[c] Chittenden, R. H., and Osborne, T. B. A Study of the Proteids of the Corn or Maize Kernel. American Chemical Journal, vol. 13, 1891, pp. 453 and 529, and vol. 14, 1892, p. 20.

Osborne, T. B. The Amount and Properties of the Proteids of the Maize Kernel. Journal of the American Chemical Society, vol. 19, 1897, p. 525.

Osborne, T. B., and Harris, I. F. The Specific Rotation of Some Vegetable Proteins. Journal of the American Chemical Society, vol. 25, 1903, p. 842.

Osborne, T. B., and Clapp, S. H. Hydrolysis of the Proteins of Maize. American Journal of Physiology, vol. 20, 1908, p. 477.

Osborne, T. B., and Harris, I. F. Nitrogen in Protein Bodies. Journal of the American Chemical Society, vol. 25, 1903, p. 323.

upon the chemistry of corn. Most investigators have contented themselves with the determination of protein, carbohydrate, fat, and ash. Some have also studied certain of the simpler constants of these groups of substances. The attempt to disentangle the mixture of complex substances of which the corn seed, like any other living thing, is composed has hardly begun. The investigators of southern Europe who had in the alleged connection between pellagra and corn a great incentive to undertake this work have not done so. In southern Europe, however, much attention has been paid to the toxicity of spoiled corn, if not to the chemistry, and the relevant literature is very large.

It is not the object of this paper to discuss the question whether pellagra is due to eating spoiled maize. The position taken is that whatever may ultimately prove to be the cause of pellagra the consumption of spoiled maize is undesirable. Even if spoiled maize should ultimately be proved to have nothing whatever to do with pellagra, its consumption would still remain decidedly objectionable for the same reasons that would apply to any other form of spoiled food. Here the economist, the hygienist, and the agriculturist meet upon common ground. If the hygienist should condemn corn as corn, it would react upon the agriculturist by narrowing the market for the country's chief crop. It is therefore of the utmost importance to the agriculturist that the deterioration of corn be investigated in all of its bearings in order that he may learn to avoid the causes of the spoiling of corn and that the consumption of spoiled corn by man may be limited. Ultimately this will be to the interest of all classes, whether growers, middlemen, or consumers. To bring about this result it must be possible to detect deterioration in corn. This is not always easy, even for unground corn. By drying moldy corn, moving it about in an elevator,[a] thereby polishing off the mold which covers the individual kernels, and by mixing it with sound corn it is possible to render the detection of spoiled corn difficult. When it is a question of meal made from spoiled corn or meal made from sound corn but spoiled after milling, the matter becomes even more difficult. In such cases special methods are necessary. Some methods for this purpose have been devised in Italy[b] and Austria.[c]

As far as known no work along these lines has been published in this country or, indeed, in the English language. To fill this gap by

[a] This process is called "running" and is a common treatment, as it aerates and thus helps to dry the corn.

[b] Antonini, G. Atti del Terzo Congresso Pellagralogico Italiano, September, 1906. Udine, 1907, p. 70.

[c] Schindler, J. Anleitung zur Beurteilung des Maises und seiner Mahlprodukte mit Rücksichst auf ihre Eignung als Nahrungsmittel. Verfaszt über Veranlassung der königlich kaiserlichen Statthalterei in Innsbruck. Innsbruck, 1909.

a critical study of the methods used in Europe and where possible to add to them is the object of the present paper. It is hoped to give criteria which will enable manufacturers of human food, public health officers, the directors of hospitals, of insane asylums, of penal institutions, and others to judge of the quality of corn and corn meal.

In Italy and Austria, where the Governments carefully control the quality of corn, suspected corn is examined by skilled government experts. In this country, where the examinations will be made in most cases only upon the initiative of private individuals, many of the tests applied abroad would often be of little service because they require a considerable degree of chemical or bacteriological skill. What seems to be needed in this country is some adequate test of so simple a character that it may be applied by the manufacturer, the health officer, or the consumer in determining whether products or purchases are fit for human food.

Such a test is thought by the writers to be the determination of the acidity of corn. This is a well-known test in both Italy and Austria, where much stress is laid on its importance. In this work it has been found the most reliable means of distinguishing good from bad corn. All corn is somewhat acid, not necessarily to the taste, but to chemical reagents. Since the spoiling of corn is due to fermentation processes in which acids are among the products, the extent to which this deterioration has progressed can be measured by the amount of acid present. It becomes necessary then only to fix a standard of acidity above which corn should be considered unfit for food.

It is desired at this point to avoid creating a misunderstanding. It is desired most carefully to avoid producing the impression that all fermented, heated, moldy, or otherwise spoiled corn is necessarily dangerous to man. This would hardly be in accord with the facts. It is, however, quite generally believed by the majority of investigators that much of this sort of corn is injurious. As long as no more definite information exists it seems the sane and conservative course to bar as far as possible damaged corn from human consumption.

The remainder of this paper will be divided into two parts, the first giving a description of the method of determining the acidity of corn and the second and longer part designed for the use of those more or less skilled in chemical manipulations and giving a critical presentation of various methods of examination.

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PART I.—METHOD OF DETERMINING THE ACIDITY OF CORN.

Apparatus necessary.

One graduated burette.

One or more 50 cubic centimeter graduated glass flasks fitted with ground-glass stoppers.

One or more 5-inch glass funnels.

One filter stand or some appliance for holding funnel while filtering.

Three-inch filter papers, preferably folded filters.

One or more 25 cubic centimeter graduated glass cylinders.

If whole corn is to be examined, a mill is necessary—a drug or coffee mill will do.[a]

Reagents necessary.

Neutral alcohol. Such alcohol may be obtained from dealers in fine chemicals. If no neutral alcohol is at hand, it may be readily prepared by the distillation of the ordinary 95 per cent alcohol with the addition of unslaked lime. A few lumps of quicklime are put in a still or retort of copper or iron; the alcohol is poured in and the still connected with a water-cooled condenser. The so-called Liebig condenser is good for this purpose. The connections may be made with suitably bent glass tubes and cork or rubber stoppers. A receiving vessel is placed under the open end of the condenser to catch the alcohol. The still or retort is then heated with a nonluminous flame till the greater part of the alcohol has boiled over. All the alcohol can not be recovered because of the danger of burning the still. An ordinary kerosene can may be used as a still, the spout of the can being connected with the condenser. If no metal vessel suitable for use as a still is at hand, a glass distilling flask may be secured from a dealer in chemical apparatus. It is best to use those made of Jena glass. The glass must not be heated directly, but must be heated over a water bath in the manner of a double boiler. To accomplish this it is immersed up to the beginning of its neck in some sort of kettle filled with water. The heat is then applied to the kettle. The flask is touched only by the boiling water. Care must be taken that the flask does not break, for then there is danger of setting the alcohol on fire. A fire of this kind is best put out by smothering it with sand, a small keg of which should be kept handy.

A solution of phenolphthalein as indicator.

Distilled water.

Twentieth normal caustic alkali (NaOH or KOH). This, too, may be purchased from dealers in fine chemicals. Only small quantities should be purchased or made at a time, as it deteriorates in a month or two, even if tightly stoppered, when it should be replaced with fresh solution.

Procedure.

If the sample to be tested is whole corn it must first be ground until all of it can be passed through the 20-mesh sieve. For this purpose a fair sample should be made, taking it from different parts of the lot—the bottom as well as the top. The sample should not be too small. It should consist of at least 500 kernels. If it is meal no further grinding is necessary, but the sample should be a mixed one, consisting of portions taken from different parts of the sack. Ten grams of the thoroughly mixed

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[a] A satisfactory mill is depicted by C. S. Scofield, in "The Commercial Grading of Corn," Bulletin 41, Bureau of Plant Industry, U. S. Dept. of Agriculture, 1903, pl. 2. For whole corn a sieve made of bolting cloth with 20 meshes to the inch will also be required. If meal only is to be examined, both the mill and the sieve may be dispensed with.

sample are weighed out and transferred to a 50 cubic centimeter graduated flask fitted with a ground-glass stopper. The flask is then filled to the 50 c. c. mark with neutral alcohol of a strength of 85 per cent by volume. After the addition of the alcohol the flasks are allowed to stand for twenty-four hours at room temperature with an occasional shaking. At the end of that period a dry filter paper is placed in the glass funnel and the stem of the funnel brought over the 25 cubic centimeter cylinder. Then the clear liquid in the 50 c. c. graduated glass flask is poured into the dry filter and collected in the graduated cylinder. When this is filled to the 25 c. c. mark, the contents are transferred to a small flask or beaker.

The alcohol adherent to the inside of the cylinder is rinsed into the beaker with a little distilled water. From 100 to 150 c. c. of distilled water and a few drops of the phenolphthalein solution are then added to the liquid. The burette, which must be clean and dry, is filled to the zero mark with the twentieth normal alkali solution and the alkali allowed to run drop by drop into the beaker, the contents of which are continually stirred, until the first permanent pale-pink coloration of the whole liquid appears. The number of cubic centimeters run into the beaker is then read off on the burette. The number of cubic centimeters twentieth normal alkali solution used, multiplied by 10, gives the acidity of 1,000 grams (1 kilogram) of corn in terms of cubic centimeters, normal alkali. The results given below under the head of acidity are calculated on this basis. It is to be noted that on the addition of the 100 to 150 c. c. of distilled water to the 25 c. c. of alcoholic extract, some zein (the alcohol-soluble protein found in corn) is precipitated, giving a cloudy appearance to the solution; but this cloudy appearance wholly or partly disappears on the addition of alkali from the burette, so that the pink coloration which marks the end point of the operation is quite obvious.

Having determined the acidity of the corn sample in terms of cubic centimeters of normal alkali, the question that next arises is whether the acidity found is that of good corn or is greater than it should be. As will be seen by reference to Part II of this paper, it has been found that the acidity number of sound corn ranges from 13 to 25; i. e., it required from 13 to 25 cubic centimeters of normal alkali to neutralize the extract from 1,000 grams (1 kilogram) of sound corn. It is necessary, however, to allow for a certain amount of variation in the corn, so that 30 cubic centimeters may be fixed upon as a safe limit. This is the limit adopted by Schindler,[a] the Austrian authority. The writers decided to calculate the acidity on a basis of 1 kilogram (2.2 pounds) to bring the figures into conformity with Fuller's scale, now very generally employed by bacteriologists.

Carried out according to this method, the determination of the acidity of corn is easily made. Any physician ought to be able to carry it out accurately, for it is far easier than to determine the acidity of gastric juice, a determination with which every physician is familiar. Graduates in pharmacy will find no trouble in performing it and it is suggested that manufacturers of human food from maize and other persons who do not wish to bother with these determinations might have them done by the local pharmacist.

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[a] Op. cit., p. 32. The writers are indebted to the article by Schindler for many valuable data incorporated in this paper.

Inasmuch as pellagra is peculiarly likely to appear in insane asylums, hospitals, and penal institutions, and inasmuch as such institutions are often compelled by law to purchase their supplies from the lowest bidder, it may be well before proceeding further to formulate rules which will enable their superintendents to specify a high grade of corn. It is advised that those purchasing corn meal for food purposes should insist that it meet the following three requirements:

(1) It shall not contain more than 12 per cent of moisture.

(2) It shall be made from degerminated corn.

(3) It shall not have a greater acidity than 30, determined by the method already detailed.

The first requirement is advised because even the best corn will spoil if it contains much moisture unless it is stored in a very cold and dry place; the second is advised because the germ, or embryo, with its high protein and fat content, is the chief point of attack by micro-organisms; the third is advised because the acidity is the simplest index of deterioration through the action of micro-organisms. All three will be discussed in detail in the second part of this paper.

PART II.—METHODS OF EXAMINING CORN.

CONDITIONS TO BE CONSIDERED.

In the examination of corn for deterioration two conditions must be considered: (1) The detection in otherwise sound corn of factors which render it liable to spoil at some future time, and (2) the detection of actual deterioration.

The detection of the former condition is very simple and consists of a determination of the moisture content, since excessive moisture content is believed to be the chief factor in causing corn to spoil.[a] Schindler[b] believes that whole corn to be safe should not contain when stored more than from 13 to 15 per cent of moisture. It is probable that in this country 15 per cent is too high a limit.

Thoroughly air-dried corn contains about 12 per cent.[c] Corn with a much greater moisture content has either been harvested too soon, as is often necessary in cold, wet seasons, or it was shelled without adequate curing on the cob. Storage under conditions which do not

[a] Scofield, C. S., op. cit., p. 20.

Duvel, J. W. T. The Deterioration of Corn in Storage. Circular 43, Bureau of Plant Industry, U. S. Dept. of Agriculture, 1909.

Shanahan, J. D., Leighty, C. E., and Boerner, E. G. American Export Corn (Maize) in Europe. Circular 55, Bureau of Plant Industry, U. S. Dept. of Agriculture, 1910.

[b] Op. cit., p. 7.

[c] Shanahan, Leighty, and Boerner, op. cit., p. 22.

protect it from the weather may, of course, increase the moisture content. Such corn is particularly liable, given a favorable opportunity, to heat and ferment.

For both whole corn and meal the drying test is the only reliable method of determining moisture and should always be applied in doubtful cases. However, for meal a different limit is required than for whole corn, since, given an equal moisture content, meal spoils more readily than whole corn. Schindler believes that 13½ per cent is the limit for meal; and that under ordinary conditions corn with a moisture content of 15 per cent will yield meal with a moisture content of 13½ per cent.[a] For this country both limits are probably too high. The actual method of carrying out these moisture determinations is so well known that it need not be described here. For the details the reader is referred to the paper of Brown and Duvel.[b]

It must, however, be pointed out that moist corn which is otherwise sound ought not to be condemned. Curing prior to storage should be insisted upon. Corn will then be in very excellent condition, fit for any use. It is perhaps worth while to point out in this connection that if growers and handlers of corn could be induced to dry corn adequately, this would result in a great addition to the wealth of the country, irrespective of any possible danger to the public health from the consumption of spoiled corn. This saving would be in at least three directions: (1) Much less good corn would deteriorate in transit and storage; (2) millions of gallons of water in the form of undesirable moisture in corn are transported annually from the corn belt; the cost of transportation of this water might be saved; (3) the germ in the corn kernel is a living thing. As long as it is not very dry it respires and gives off carbonic acid and water. Like all living things it uses up food in the process of respiration. The food it consumes is the material stored in the endosperm. It is clear that the more food the embryo respires away the less will be left for man. Now, it has been proved that the drier corn is the less it respires, until, as it approaches absolute dryness, respiration becomes minimal.[c] It is evident, then, that moist corn must lose in food value in the course of time more than dry corn. It is impossible at present to say exactly what this loss amounts to, because data on the variation of respiration with moisture content do not exist.. It is probably not great enough to affect seriously any single owner of corn, but it is quite probable that if it were possible to cal-

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[a] Op. cit., p. 24.

[b] Brown, Edgar, and Duvel, J. W. T. A Quick Method for the Determination of Moisture in Grain. Bulletin 41, Bureau of Plant Industry, U. S. Dept. of Agriculture, 1907.

[c] White, Jean. The Ferments and Latent Life of Resting Seeds. Proceedings of the Royal Society, vol. B 81, p. 417.

culate it for the country as a whole it would amount to a very large sum indeed.

The method of detecting actual deterioration of whole corn differs from that for corn meal. The methods for each will therefore be considered separately.

EXAMINATION OF WHOLE CORN BY INSPECTION.

Good corn must be sufficiently dry, as has been discussed above. It must be mature. It should not contain many cracked, rifted, or broken kernels. The hull protects the kernel from the attacks of bacteria and fungi. If the hull is burst or the kernel broken, the grain is likely to become moldy. The rifts may be due to imperfect artificial drying or to the careless shelling of inadequately cured corn. However, care must be taken not to confuse rifts of this type with the small ones, which are entirely internal, due to shrinking of the horny layer. The latter do not penetrate the hull, and therefore are unobjectionable, because they do not give access to micro-organisms. They are due to artificial drying at too high a temperature or more frequently to drying very moist corn too rapidly. When grain is observed to be covered with white powder, it has probably been damaged by insects, the granary weevil (*Calandra granaria* L.), the rice weevil (*Calandra oryza* L.), the wolf moth (*Tinea granella* L.), the Angoumois grain moth (*Sitotroga cerealella* Ol.), or other insects.[a] Injury by insects is of importance for the same reason that a burst hull is. By piercing the hulls insects open the way for fungi. Good corn should not contain many moldy or bad kernels. Schindler[b] believes that a content of more than 5 per cent of them should not be allowed. This limit is probably a good one when the grain is examined in the laboratory in the careful way advised in this paper. When, however, the grain is examined in the usual way by the grain inspector, only the more seriously damaged kernels would be apt to be noticed, so that under these circumstances this limit is probably too high. Under these conditions 2 to 2.5 per cent of moldy or cob-rotten kernels is a safer limit.[c]

The mold or bacterial growth may be either superficial, the fracture surfaces of broken kernels being attacked with particular frequency, or it may be in the interior when this has become accessible as the result of cracks, rifts, or injury by insects. It is then almost always the embryo which is the site of the growth of micro-organisms, presumably because it presents the most favorable soil. Sometimes

[a] For details the reader is referred to "Some Insects Injurious to Stored Grain," by F. H. Chittenden, Farmers' Bulletin 45, U. S. Dept. of Agriculture, 1897.

[b] Op. cit., p. 15.

[c] See Shanahan, Leighty, and Boerner, op. cit., p. 42.

this growth is evident only as a faint, bluish-gray spot, barely perceptible through the hull covering the groove in which the embryo lies. It is easily overlooked by the inexperienced, and it is therefore wise to trim off with a small sharp-pointed knife the hull covering the groove of suspicious-looking kernels, when the sound or decayed condition of the embryo may be recognized by anyone. If the decay is more advanced, the embryo may appear distinctly bluish-green, and when the hull is removed it will be seen that the embryo has been more or less completely replaced by a bluish-green powder, the spores of the fungi. Such grain is often known as blue or black eyed corn. In extreme cases the entire surface of the kernels may be covered with this bluish-gray or greenish mold powder. This discoloration seems to be caused by members of the genus of molds known as Penicillium. Other molds will produce other shades of color. One sample of corn examined in the course of the present investigation was covered with a bronze-colored powder. Dr. Erwin F. Smith, of the Bureau of Plant Industry, who examined it, identified it as spores of *Aspergillus fumigatus*. Doctor Duvel in a personal communication states that he has not infrequently encountered corn spoiled in this way. It is stated that sometimes the embryo is colored reddish by *Micrococcus prodigiosus*. In deciding whether any given kernel is moldy or not, one must be careful not to be misled by the color of the tip cap, which is often naturally of a darker color than the rest of the kernel.

Corn which has heated in bulk may show the result of bacterial action rather than that of molds. It is often more or less irregularly discolored, showing lighter and darker blotches and streaks, more especially in the region of the embryo and toward the tip. These spots are colonies of micro-organisms which are not merely confined to the surface, but also invade the interior of the kernel. In extreme cases the heat developed may be so great that the corn becomes brown or black and charred.

Good corn, finally, should have the fragrance characteristic of good meal. Spoiled corn has sometimes a musty or a sour odor, which may be intensified by warming it slightly in some way, such as holding it for a few moments in the closed hand or by blowing the breath upon it. Good corn should have the characteristic, slightly sweet taste of good meal. Spoiled corn may lack this characteristic taste and is often bitter.

These are the external criteria by which corn may be judged in regard to its fitness for human food. Their practical application in examining corn will now be considered. The first point is to obtain a fair sample. As already indicated, samples should be taken from various parts of the mass of corn; from the top, the bottom, and

different levels between, and from the sides. The number of samples to be taken will depend upon the quantity of corn. Whether the odor be musty, or sour, or like the interior of a silo is noted as each sample is taken. The general appearance of each sample must be observed, for in dealing with large masses of grain different conditions may be met with in different regions of the mass. If this proves to be the case, the different samples are best examined separately. Ordinarily, however, the various samples are thoroughly mixed and the sample for examination taken from the mixture at several different points. The moisture content is determined accurately.

The pile is then spread out in a thin layer and the corn examined to see whether it is of characteristic bright, shiny appearance or whether the kernels are dull, blotched, discolored, with colored embryo indicative of heating and fermentation, or whether they are pale and shriveled, sometimes indicative of immaturity. The presence of many rifted, broken, or cracked kernels, or of much foreign matter, such as weed seeds or such débris as pieces of cob, is noted. While the latter are not in themselves necessarily harmful, they are hotbeds of molds which are liable under favorable conditions to infect the sound kernels.[a] A large number of kernels are next examined, one by one, for insect injury, and with a sharp-pointed knife the hull is removed from the embryo to show whether its condition is good. By this superficial examination an idea is obtained of the number of spoiled kernels present, which if excessive must be determined.

To do this, small numbers of kernels from different parts of the sample as it lies spread out thin on the white paper are taken until there are at least 500 kernels. These are spread out on white paper and each kernel examined individually, the good being put in one pile and the bad in another. When all have been examined each pile is weighed and the percentage of spoiled kernels computed. This should not exceed 5 per cent.[b]

BIOLOGICAL EXAMINATION.

The biological examination of corn was first proposed by Sclavo.[c] It is based on the fact that the chief point of attack for micro-organisms is the embryo, or germ. If the action of the micro-organisms is enough to kill the germ, the kernel loses its power to germinate. The best seed corn germinates as high as 97 per cent

[a] See Shanahan, Leighty, and Boerner, op. cit., p. 23.

[b] See p. 14.

[c] Sclavo, Vincenzo. Gazzetta Medica di Torino, vol. 52, October 24, 1901, p. 853.

and over.[a] The method of determining germination is very simple. For details the reader is referred to the paper of Hartley.[a] It is only necessary to add that at least 100 kernels should be tested. No tests were made upon commercial grades of corn in the work here reported, and therefore a standard can not be fixed. The Italian Government[b] has fixed as a limit a germinating power of 80 per cent, while Ori[c] protests that this limit is too low. He advocates a limit of 90 per cent. This test, simple and excellent though it be, is not universally applicable. If perfectly sound but moist grain be dried at too high a temperature, the germinating power may be destroyed though the grain be of excellent quality. This is not likely to happen in the United States, for the driers do not ordinarily work at a sufficiently high temperature. Indeed, it is stated in a personal communication by Doctor Duvel, of the Office of Grain Standardization of the Bureau of Plant Industry, that he has known moist corn to gain in germinating power by being passed through a drier.[d] Furthermore, if corn of very high germinating power were mixed with spoiled corn of very low germinating power, this admixture might escape detection though it exceeded 5 per cent, because the germinating power might still exceed 90 per cent.

It may be well, apropos of the dependence of the biological test upon the sound condition of the embryo, or germ, to point out the importance of the germ in determining the quality of the manufactured meal. As already indicated, the germ is the chief site of attack by micro-organisms. By removing the germ from corn that has not been too badly spoiled the greater part of the micro-organisms and their products will be removed. If the statements of European investigators concerning the toxicity of spoiled corn are to be believed, it follows that degerminated spoiled corn is less toxic than it was before the removal of the germ. Indeed, it has been shown that in the process of milling the more unwholesome material goes into the poorer grades of meal, which contain the starchy part of the endosperm lying next to the germ, and also into the germ,[e] which in this country is used for the manufacture of corn oil and stock feed. Moreover, the high oil content of the germ renders meal from whole corn less desirable than that from degerminated corn, since

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[a] See Hartley, C. P., "The Production of Good Seed Corn," Farmers' Bulletin 229, U. S. Dept. of Agriculture, 1905, p. 19.

[b] Rivista Pellagrologica Italiana, vol. 5, p. 122.

[c] Ori, A. La Diagnosi delle Alterazioni del Maiz in Chicchi ed in Farina. Rivista Critica de Clinica Medica, 1906, p. 165.

[d] See Webber, H. J., in appendix (p. 22) to paper of C. P. Hartley previously cited.

[e] Balp, S. Venticinque Anni di Lotto contra la Pellagra (1881–1906), Biella, 1908.

the oil is likely to become rancid. These are the reasons why in the foregoing part of this paper the advice was offered that meal from degerminated corn should have preference over that from whole corn. These considerations also render it likely that lye hominy is a wholesome form of corn, for the treatment with lye not only removes the hulls and germ but destroys micro-organisms. The method of determining whether meal has been made from whole or degerminated corn will be given later in discussing the chemical methods of examination.

The methods hitherto presented, namely, the determination of acidity, moisture, and germinating power, and the examination by inspection, are adequate for the examination of whole corn. Only the first two are, however, applicable to meal. These are chemical methods, and chemical methods are relied on mainly in dealing with meal.

CHEMICAL METHODS.

SIGNIFICANCE OF THE ACIDITY DETERMINATION.

The most important and the most universally applicable chemical method is the determination of acidity. This has already been adequately treated, but the analytical data on which the writers base their procedure and their estimate of its value will be given.

The effect of fineness of grinding upon the determination has been investigated. It has been found that when corn is ground fine enough to pass through a sieve with 16 meshes to the inch, only a little is gained by making the grinding finer, as shown in Table I. The difference between a sample that had passed through a 16 mesh and the same sample passed through a 24 mesh was only half a cubic centimeter of the alkali. Twenty meshes to the inch has therefore been fixed upon by the writers as a convenient standard. Most commercial meals will pass through such a sieve.

TABLE I.—*Relation of fineness of sample and length of extraction to the acidity determination.*

Determination.	Ground to 16 mesh; stood 24 hours.	Ground to 24 mesh; stood 24 hours.	Ground to 16 mesh; stood 5 hours; infrequent shaking.	Ground to 16 mesh; stood 5 hours; frequent shaking.
	c. c	*c. c.*	*c. c.*	*c. c.*
Acidity	36.5	37.0	32.5	34.5

An endeavor was also made to shorten the time of extraction. The acid from the meal passes very slowly into the alcohol. Even after twenty-four hours the extract has not attained the maximum acidity. The figures show, however, that extraction for twenty-four hours gives uniform and comparable results, and this is all that is

necessary for practical purposes. Extraction for five and seven hours gives values too low (Table I). For reasons of practical convenience, therefore, twenty-four hours has been fixed upon by the writers as the standard time of extraction, even though it will not record the maximum acidity. As long as the determination can not be finished within the eight hours of the working day there is no object in making the extractions less than twenty-four hours. Warming the flasks hastens the extraction, but is objectionable because it causes much zein to go into solution. Moreover, at the higher temperatures a variation of a few degrees makes far more difference than in a determination at room temperature. This would introduce a source of error unless a thermostat were used; further, the use of heat with or without a thermostat would complicate the method. It is possible to shorten the time of extraction by vigorous shaking (Table I). In the regular determinations the flasks were merely turned upside down several times, three or four times the first eight hours, and once a little while before the titration. This shaking by hand occupied but a few moments. During the remainder of the time the flasks stood quietly at room temperature. By the use of a shaking machine the time of extraction could no doubt be shortened very much. Should this determination ever come into general use, large establishments testing many samples daily could use a shaking machine with profit. A machine was not used in the present investigation, because it would complicate the procedure and because each machine would have to be adjusted for a definite set of conditions.

The effect upon the acidity determination of slight changes in the concentration of the alcohol has also been examined. These have to be considered because of the variation in moisture content of corn samples. This moisture would dilute the alcohol and might introduce an error. Acidity determinations were therefore made with 80 per cent as well as with 85 per cent alcohol. The moisture would never be likely to be sufficient to lower the strength of the alcohol from 85 per cent to 80 per cent. Determinations were made upon a sample of spoiled meal that was very acid and upon a sample of good meal. The results are presented in Table II. The differences due to the variations in concentration of the alcohol obtained are insignificant.

TABLE II.—*Relation of alcohol concentration to the acidity determination.*

No. of sample.	80 per cent alcohol.	85 per cent alcohol.
12	76 c. c. N. alkali.	78 c. c. N. alkali.
16	22 c. c. N. alkali.	22 c. c. N. alkali.

Table III is a presentation of the acidity of a number of samples of seed corn of various strains from various parts of the country. Mr. C. P. Hartley, Physiologist in Charge of Corn Investigations, Bureau of Plant Industry, furnished most of the samples. The determinations were made in February, 1910, except No. 10, which was made in December, 1909. The samples contain specimens of the crop of 1909 and 1908. It is seen that the acidity ranges from 13 to 24 c. c. and that the 1908 corn is no more acid than that of 1909. No. 51 is from the same ears as No. 50; it differs from the latter in consisting only of the smaller kernels from the tips of the ears. No. 28 is corn specially bred for low-protein content, while No. 29 was specially bred for high-protein content. No. 40 is corn prematurely ripe, such as is often produced in years with exceptionally warm and dry autumns.

TABLE III.—*Acidity and moisture of selected samples of high-grade corn from various sections of the United States.*

No. of sample.	Name of variety.	Ash.	Acidity.	Moisture.	Locality.
		Per cent.	*c. c.*	*Per cent.*	
1	Sturgis Hybrid, 1908	1.59	18.0	7.60	Connecticut.
2	White North Dakota Flint, 1908	1.22	18.0	8.11	North Dakota.
3	Barnwell White, 1908	1.25	17.5	7.93	South Carolina.
4	Marlboro Prolific, 1908	1.25	13.0	7.71	Do.
5	Boone County White, 1908	1.29	15.0	7.47	Tennessee.
6	Huffman, 1908	1.42	13.0	8.25	Do.
7	Strawberry, 1909	1.55	18.0	7.56	Texas.
8	Marlboro Prolific, 1909	1.38	15.0	10.09	South Carolina.
9	Boone County White, 1909	1.23	13.0	7.81	Tennessee.
10	Whole corn, selected ears, 1909	1.18	23.9	10.00	Maryland.
28	Low-protein corn		16.5		Illinois.
29	High-protein corn		19.5		Do.
40	Prematurely ripe white corn		24.0		District of Columbia.
50	Whole seed corn		16.2		Virginia.
51	Corn tips		16.5		Do.

Small samples of meal were purchased in the open market in Washington, D. C., Summerville, S. C., Boston, New York, and Chicago, In all the cities but Washington the samples were purchased from little stores in the parts of town where poor people trade. In Washington the samples were purchased in different parts of the city, in the fashionable residential section as well as the poor quarters. The results are presented in Table IV. This table also includes meal No. 11, ground in the laboratory from corn which had been allowed to spoil in the bin of a grain elevator at Baltimore during the course of an experiment conducted by Doctor Duvel,[a] who very kindly furnished not only this sample but also many others. Doctor Duvel and Mr. Shanahan, the Crop Technologist in Charge of Grain Standardization, Bureau of Plant Industry, gave much help and

[a] See Duvel, J. W. T., "The Deterioration of Corn in Storage," Circular 43, Bureau of Plant Industry, U. S. Dept. of Agriculture, 1909.

advice. Nos. 12 and 14 were from two institutions in which cases of pellagra had occurred. It will be seen that a considerable number of samples have too high an acidity.

TABLE IV.—*Acidity of samples of commercial corn meal purchased in several cities in the United States.*

No. of sample.	Variety.	Water.	Ash.	Acidity.	Locality.
		Per cent.	*Per cent.*	*c. c.*	
11	Whole corn, spoiled	9.5	1.50	37	Baltimore.
12	White meal	10.0	1.04	78	Illinois.
14	.....do	10.7	2.00	60	Arkansas.
15	.....do	10.0	1.47	33	Washington, D. C.
16	Yellow meal	10.8	.33	23	Do.
17	White meal	10.4	1.03	39	Do.
18	Yellow meal	10.0±	.22	17	Do.
19	White meal	10.0±	1.08	29	Do.
20	.....do	10.0±	1.06	29	Do.
21	.....do	10.0±	1.30	41	Do.
22	.....do	10.0±	1.05	....	Do.
23	.....do	10.0±	1.26	37	Do.
24	.....do	10.0±	.98	24	Do.
30	.....do	....	....	23	Summerville, S. C.
31	.....do	....	....	28	Do.
32	.....do	....	....	16	Do.
33	.....do	....	....	30	Do.
34	Yellow meal	....	....	37	Boston.
35	.....do	....	....	37	Do.
36	.....do	....	....	35	Do.
37	.....do	....	....	19	Chicago.
38	.....do	....	....	20	Do.
39	.....do	....	....	23	Do.
41	.....do	....	....	19	New York.
42	.....do	....	....	23	Do.
43	.....do	....	....	40	Do.
44	.....do	....	....	21	Do.
45	.....do	....	....	18	Do.
46	.....do	....	....	16	Do.
47	.....do	....	....	13	Do.
48	.....do	....	....	29	Do.
49	.....do	....	....	17	Do.

The mother substances of the acid formed have not yet been finally determined, but some of the analytical results give indications as to their nature.

TABLE V.—*Analyses of different portions of a carload of damaged corn.*

Sample.	Water.	Ash in dry material.	Acidity.	Fat in dry material.	Nitrogen in dry material.
	Per cent.	*Per cent.*	*c. c.*	*Per cent.*	*Per cent.*
No. 25, top	11.53	1.51	95.0	4.25	2.53
No. 26, 2 inches from top	8.33	1.41	73.0	3.94	1.86
No. 27, 6 inches from top	8.06	1.24	64.0	3.87	1.29
Normal corn	[a] 10.75	[a] 1.50	[b] (15–30)	[a] 4.2	[a] 1.60

[a] Wiley. [b] Schindler.

Table V gives analyses of three samples of corn taken from the same car while undergoing heating. No. 25 was taken at the surface, No. 26 was taken 2 inches below, and No. 27 was taken 6 inches below the surface. Appended to Table V is an analysis of average corn

published by Wiley.[a] No. 25 had sprouted but had been killed before growth had advanced beyond a beginning. It was covered with blue-green mold and had a very musty odor. To the eye, nose, and tongue it was one of the worst specimens handled. No. 26 was less moldy but still had a musty odor blended with a sour smell. No. 27 was characteristic of heated corn and had a very sour smell.

These differences may be due to the fact that on the surface corn the aerobic fungi flourished, while down in the interior the anaerobic ones developed. It must, however, be remembered that scientists are at variance as to the mechanism which causes the heating of vegetable material when it is bulked. There are three views. Some believe that the heating is due in the main to bacterial action.[b] Others believe that it is due to the action of oxidizing enzymes.[c] Finally, Boekhout and Ott de Vries[d] have shown that oxidation can take place by simple catalysis under conditions which exclude the intervention of micro-organisms as well as enzymes. No similar studies have been made upon corn or, indeed, upon any other seed, so that as yet it can only be surmised what takes place in these cases. It will be seen that No. 25 with the highest acidity has also the highest fat and nitrogen content. This is not due to an absolute increase in these substances but to a relative one caused by the disappearance of some other substance which can not be anything other than carbohydrate. Here is evidence, then, that carbohydrate, in this single case at any rate, furnished the material from which acid was formed. This is in accord with what is known in general about fermentation and with the observations of Italian authors.[e]

It is, of course, probable that the fat is more or less saponified, thereby becoming rancid, and that the fatty acids formed contribute to the acidity. This point is being investigated by the writers. It is particularly important in the light of recent researches upon the toxicity of unsaturated fatty acids.[f]

The figures of Table V are perhaps not typical of all cases of spoiling. It is even probable that under different conditions quite a

[a] Wiley, H. W. Composition of Maize. Bulletin 50, Bureau of Chemistry, U. S. Dept. of Agriculture.

[b] Miehe, H. Die Selbsterhitzung des Heus. Jena, 1907.

[c] Loew, O. Curing and Fermentation of Cigar-Leaf Tobacco. Report 59, U. S. Dept. of Agriculture, 1899.

[d] Boekhout, F. W. J., and Ott de Vries, J. J. Über Tabaksfermentation. Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, vol. 24, pt. 2, p. 496.

[e] Gosio, B. Ricerche Batteriologiche e Chimiche sulle Alterazioni del Mais. Contributo all' Etiologia della Pellagra. (Memoria 2 a) Rivista d'Igiene e Sanità Pubblica, vol. 7, 1896, p. 825.

[f] Faust, E. S. Über chronische Ölsäurevergiftung. Archiv für Experimentelle Pathologie und Pharmakologie. Supplement, 1908, p. 171.

different state of affairs may be found. These figures are to be regarded only as a single instance until more samples of a similar nature are analyzed.

The writers have begun investigating the nature of the substances which render the extracts acid. This seemed important because zein might be one of them. Zein is one of the chief proteins of the endosperm. It probably does not occur at all in the embryo before germination.[a] It is soluble in moderately strong alcohol, insoluble in dilute and absolute alcohol, and behaves somewhat like an acid in combining with a certain amount of alkali. Its solubility in alcohol insures its being present in the extracts. As it is more soluble in hot than in cold alcohol it seems possible that differences in the temperature of the room during extraction might cause more or less of it to pass into solution and thus affect the results. It was therefore important to determine whether the increased acidity of spoiled corn was dependent to any considerable degree upon the zein. To settle this point both the acidity and the nitrogen content of the extract of both spoiled and sound corn was determined. The nitrogen content would give an index of the amount of zein that had gone into solution. The figures obtained are given in Table VI. Under the heading "Kjeldahl nitrogen" is given the amount of nitrogen, as determined by the Kjeldahl method in the amount of alcoholic extract used for the titration (25 c.c.). The same extract used in the titrations was used for the nitrogen determinations. Hence the acidity and the nitrogen figures are quite comparable.

TABLE VI.—*Relation between the total nitrogen and the alcoholic extract of corn meal.*

No. of sample.	Kjeldahl nitrogen.	Acidity.
	Grams.	*c. c.*
14	0.0139	60.0
10	.0297	23.0
25	.0099	95.0
26	.0140	73.0
27	.0105	64.0
28	.0081	16.5
29	.040	19.5

It will be seen that the very acid extract from the spoiled corn contains less nitrogen and therefore less zein than the extract from good corn except sample No. 28. Now No. 28 is a low-protein corn. Its acidity is low and the small amount of nitrogen in the extract seems to depend not on the acidity but upon the low-protein content of the grain. This assumption gains in probability by the figures obtained

[a] Soave, M. L'Azoto della Zeina in Relazione all' Azoto Totale e all' Azoto delle Altre Sostanze Proteiche nel Mais. Le Stazioni Sperimentali Agrarie Italiane, vol. 40, p. 193.

with No. 29, which is a high-protein corn. This gives the highest nitrogen figure and yet differs in acidity by only 3 c. c. from No. 28, which gives the lowest nitrogen figure. This difference in the behavior of low and high nitrogen corn is very suggestive from a number of points of view. From the present standpoint it is of interest that although the amount of nitrogen in the alcoholic extract probably influences to a slight degree the acidity values obtained under the present conditions it can not possibly influence them enough to invalidate the usefulness of the acidity determination. Furthermore, in sound corn the acidity of the extract can depend only partly upon zein, and in spoiled corn it can depend only to a slight degree upon it.[a] As a matter of fact it would seem that less nitrogen is extracted when the acidity is high. The question arose whether the lessening of the nitrogen extracted is due to a consumption of zein by the micro-organisms causing the deterioration or whether acidity renders zein less soluble.

To test this, good corn was extracted with neutral alcohol in the usual way and also with alcohol to which sulphuric acid had been added until its acidity corresponded to that of spoiled corn. The result did not come up to expectations, for the differences were insignificant as the following figures show:

Extraction with neutral alcohol and with acidified alcohol.

Sample 16.	Extracted by 85 per cent alcohol.	Extracted by 85 per cent alcohol+5 c. c. H_2SO_4 N/10.
	Grams.	*Grams.*
Kjeldahl nitrogen	0. 0241	0. 0221

These negative results, together with the figures obtained for low-protein (No. 28) and high-protein (No. 29) corn, make it probable that it is not the acidity developed but the destruction of alcohol-soluble nitrogenous material which accounts for the low-nitrogen content of the alcoholic extracts of very acid corn.

The conversion of the corn protein into other substances was a matter which gave considerable food for thought. As long as it was not known that zein was an unimportant factor in causing the acidity of the extracts it was deemed possible by the writers that altered zein might be the cause of the acidity. As already pointed out, zein behaves in some respects like an acid. The putrefaction of proteins is accompanied among other processes by their deamidization, i. e., the removal of ammonia, either as such or in the form of amins. The removal of

[a] Prof. L. H. Smith, of the Illinois Agricultural Experiment Station, kindly furnished samples of low-protein and high-protein corn.

these basic groups might increase the acid properties of the proteins. It was possible that zein might in this way become more acid without sacrificing its solubility in alcohol. However, when it was found that the acidity did not depend to any large degree upon zein the investigation of this point was postponed to some future time. For the present the fact is noted that the zein from spoiled corn was found to be different from that prepared from sound corn. When freshly precipitated it is not so tough, but rather brittle and of a dirty green color, even when obtained from white corn. It could not be decolorized with any of the ordinary fat solvents; a study of the nature of the change is now being made.

These considerations suggest the possibility of corn spoiling so as to become alkaline from the formation of ammonia and amins. This would of course ultimately take place, but probably not until all the starch in the grain had been used up. When it did occur decomposition would be so far advanced that use of the corn as food would be quite out of the question. The possibility of ammoniacal decomposition does not therefore vitiate the acidity test.

The nature of the acids formed has also been studied by the writers. This seems to be an intricate question, since it involves more than a simple acetic or lactic acid fermentation. A number of interesting results have been obtained which it is hoped to communicate at a future time. For the present the fact is recorded that a peculiar volatile crystalline acid has been encountered which could not be identified as any of the ordinary fermentation acids. It is possible that it is identical with the acid isolated by Gosio,[a] and it is hoped that its identity may be learned.

ASH DETERMINATION.

The ash determination is done in the usual manner. However, corn is quite difficult to ash without employing temperatures so great that there is danger of loss by volatilization. Experience has taught that the following manipulations are useful. Porcelain is best used, as platinum is badly attacked. The heating is begun with a very small flame, and at least half an hour is allowed for the material to become charred. In this way a porous mass is obtained. Rapid heating causes the meal to char and covers it with a coating of fused salts which effectively keeps the oxygen from gaining access to the carbon. In the course of an hour or two the flame is gradually raised to the full heat of an ordinary Bunsen burner. When after a time the carbon does not seem to be disappearing, the crucible is cooled and water added. This water is then evaporated off on the steam bath. The pieces of carbon float on the surface and climb up the sides of the crucible, so that when the crucible is dry and is again heated they

[a] Op. cit., p. 871 et seq.

burn off readily. Sometimes a second treatment with water is necessary.

In Italy the amount of ash present is regarded as significant. An ash content of over 4 per cent is considered a sure sign of deterioration.[a] Undoubtedly it is. Fermentation increases the ash content because the fungi causing the fermentation consume organic matter in the corn kernel, converting it into carbonic acid and water, which are dissipated into the atmosphere. None of the salts disappear. Consequently, since the organic matter in the fermented kernel is lessened, the relative proportion of salts and similar constituents is increased and the percentage of ash rises correspondingly. An inspection of Table III shows that the ash content of good corn can be considered as being in the neighborhood of 1.5 per cent. Inspection of Tables IV and V shows further that badly spoiled corn (Nos. 11, 25, 26, and 27) does not necessarily have a very high ash content. Only in meal No. 14 is it noticeably high. Evidently conditions are different in Italy or else corn far more badly spoiled than any seen in the course of this investigation is common. There was no sample with more than 2 per cent of ash, yet Tables IV and V show that in a general way ash content and acidity run parallel. The ash determination is troublesome, the acidity determination easy. Therefore in most cases the former may be omitted.

From another point of view the ash determination is significant. It gives an indication as to how completely a meal has been degerminated and the starchy layer of the endosperm removed. Nearly all the ash of the kernel is located in the germ. Hence, the poorer the meal in ash the more complete the removal of the germ and the adjacent starchy layer. How desirable it is to degerminate corn has already been shown. This is again expressed in the ash and acidity determinations of Table V. Thus, the meals most completely degerminated, those with the lowest ash content, show also the lowest acidity. Nos. 16 and 18 were yellow meals milled for the northern market and consisted almost exclusively of the horny layer of the endosperm. The very fact that American meals vary so much in the degree of degermination renders ash determinations an unsatisfactory method for the examination of meal. Thus, meal made from thoroughly degerminated corn would have a low ash content. Subsequently, owing to moisture or faulty storing, it might become very bad indeed without showing an ash content as high as that of meal from whole corn.

FAT DETERMINATION.

Although the ash determination gives an index of the degree of degermination of a meal, this can be estimated more accurately by a

[a] Antonini, G., op. cit., p. 74.

fat determination. The germ contains only 10 per cent of ash, but it has often over 30 per cent of oil. Consequently, the fat determination is the more delicate index of the two. Whole corn contains on the average about 4.3 per cent of fat. High-grade, rather coarse meal, consisting only of the horny layer, may contain as little as 0.8 per cent of fat. Meals on the average will vary between these limits according to the degree of degermination. In one other direction the fat determination is useful. It makes it possible sometimes to determine whether a meal has been adulterated with the germ. No such case has been met with in the present research, but it seems to have been attempted in Europe. Millers have there adulterated their low-grade meals with the germ obtained as a by-product in the manufacture of their high-grade meals. Such adulterated meal will of course show a fat content high above that of whole corn.

The fat determinations are carried out in the usual way with a Soxhlet extractor.

THE PHENOL REACTION OR TEST OF GOSIO.

In Italy much stress is laid upon the phenol reaction. Schindler [a] discards it as uncertain; it could be obtained only once, in sample No. 25, the worst one dealt with. This fact strengthens the suspicion that corn as bad as that which seems to be common in Italy is rare in this country.

The phenol test depends upon the formation by molds of substances giving color reactions with ferric chlorid. The Penicillium molds, or at least some of them, are said to produce this substance or substances. Gosio [b] has endeavored to isolate the substance. He obtained a small amount of a crystalline substance giving a color with ferric chlorid, possibly parahydrocumaric acid. It was not toxic. Gosio,[b] Gosio and Ferrati,[c] and Antonini and Ferrati [d] all believe that the toxic substance and the substance giving the color with ferric chlorid are identical. They believe that the toxicity and the reaction of Gosio run parallel. These views are not accepted by all Italians and have been particularly vigorously attacked by Ceni.[e] Most Italian investigators believe this reaction to be caused by phenols

[a] In a personal communication.

[b] Op. cit., p. 869 et seq.

[c] Gosio, B., and Ferrati, E. Sull' Azione Fisiologica dei Veleni del Mais Invaso da Alcuni Ifomiceti. Rivista d'Igiene e Sanità Pubblica, vol. 7, 1896, p. 961.

[d] Antonini, G., and Ferrati, E. Sulla Tossicità del Mais Invaso da "Penicillium glaucum." Archivio di Psichiatria, Scienze Penali ed Antropologia Criminale, vol. 24, p. 581.

[e] Ceni, C. Sulla Reazione Fenolica in Rapporto coi Tossici Pellagrogeni. Rivista Pellagrologica Italiana, vol. 6, 1906, p. 60.

or phenol acids. This belief is based not upon the isolation and chemical identification of these substances, but upon the ferric-chlorid reaction and the fact that extracts giving this reaction kill mice with symptoms resembling carbolic-acid poisoning. When it is considered how many substances give color reactions with ferric chlorid and, further, how difficult it is to form any opinion of the identity of a poison from the symptoms it produces in animals, it must be concluded that it is premature to pass judgment on the chemical nature of these substances.

In its original form the reaction of Gosio is performed in either of the following ways:

(*a*) From 50 to 100 grams of meal are warmed for several hours in twice their volume of 80 per cent alcohol. The alcohol is then filtered off into a porcelain dish and evaporated to dryness. The residue is then taken up with warm water, filtered, and the filtrate treated with a dilute solution of ferric chlorid. A coloration varying from dark green to bluish violet results.

(*b*) The meal is suspended in water acidified with a few drops of phosphoric acid. The acid suspension is exhausted with ether, the ethereal extract evaporated to dryness, and the residue tested as above.[a] Antonini[b] advises that if the first-mentioned procedure is followed the extraction be continued for several days, shaking from time to time and exposing to the sunlight. In order to avoid resins and fats which may obscure the reaction, the residue may be extracted with boiling water, the extract filtered, and the filtrate tested. If the second procedure is followed, he advises using three times as much 1 per cent phosphoric-acid solution as corn (by volume) for the extraction, and he prolongs it for several days, shaking thoroughly, exposing to sunlight, and warming slightly. The writers attained the best success with this modification. When the extraction has gone on long enough, the suspension is cooled, and then treated with two to three volumes of ether. This is allowed to separate and the clear ether, which alone should be used, is decanted. It is shaken out repeatedly with distilled water to remove impurities. Finally, the clear ether is decanted from the water, distilled off, and the residue tested.

According to Antonini, Camurri has modified the test of Gosio by distilling the meal with water or steam and performing the reaction upon the distillate. The reaction is said to be even more distinct if it be performed upon the ethereal extract of the distillate.

THE REACTION OF ORI.[c]

The reaction of Ori depends upon the fact that molds contain or produce a substance or series of substances which decompose per-

[a] Gosio, B., op. cit., p. 883. [b] Op. cit., pp. 74–75. [c] Ori, A., op. cit.

oxid of hydrogen catalytically. The substance producing this decomposition is believed to be an enzyme and has been called catalase. It is probably of universal occurrence in living things and therefore also occurs in the corn kernel. However, it seems to be more abundant in molds than in corn. Consequently, moldy corn or moldy meal will decompose peroxid of hydrogen more powerfully than good corn or good meal. The reaction is carried out as follows:

Five grams of meal are extracted for half an hour with 15 c. c. of a 50 per cent aqueous solution of glycerin. The extract is then filtered through paper; 1 c. c. is put in a watch glass and 4 to 5 drops of a 3-per cent peroxid of hydrogen solution added. Good degerminated meal gives no bubbles at first, while bad meal produces a strong effervescence almost at once.

The writers conclude that in general this reaction gives a good indication of the condition of the meal if the meal be thoroughly degerminated. As Ori himself points out, the reaction is more reliable than that of Gosio, while, as his figures show, it runs parallel with the acidity. Judged by his figures, it does not seem to be more delicate. Now, good corn kernels, as already stated, contain a certain amount of catalase, and therefore meal made from whole corn decomposes peroxid of hydrogen to a certain extent. Usually, however, this is not as extensive as when the corn is moldy. The writers found by taking corn kernels, splitting them, paring the germ carefully away, and making extracts separately of the endosperm and the germ that the catalase is located almost exclusively in the germ.[a] The extract of the germ gives practically as powerful a reaction as spoiled meal. Here, then, are possibilities of confusion. Thoroughly degerminated meal ought not to decompose peroxid of hydrogen. Meal from good whole corn will decompose peroxid of hydrogen to a certain extent. Hence, it is conceivable that meal from very thoroughly degerminated corn may become somewhat moldy and yet give Ori's reaction no more intensely than meal from good whole corn. Therefore, in order to form a correct estimate of the value of the reaction in any given case it ought to be known whether the product was obtained from degerminated material. Viewed from this aspect the reaction of Ori has its value. On the other hand, there is another possibility. It seems conceivable that meal might be made from corn spoiled in such a way that the molds were situated mainly in the germ. If in the process of milling the corn were thoroughly degerminated and carefully bolted, the greater part of the molds might be removed.

[a] Since making these experiments it was discovered that similar observations upon wheat have been made very recently by P. Liechti. See Die Prüfung von Mehlen auf Grund ihres Gehaltes an Katalase, Vorläufige Mitteilung, Chemiker Zeitung, vol. 33, p. 1057.

In such a case the corn might show a fairly high acidity and nevertheless a weak reaction of Ori. Meal with high acidity and negative action upon peroxid of hydrogen was actually encountered by Ori, and he points out that this phenomenon might in some way be connected with degermination.[a] There is still another factor to be taken into consideration. Catalase is an enzyme. It is therefore weakened or destroyed by temperatures of 60° C. and higher. Artificially dried corn might, therefore, when carelessly dried, lose its power to decompose peroxid of hydrogen.

It is quite possible that with these limitations this reaction might be developed into a useful rapid method if it were made quantitative. This ought to be easy, either by measuring the volume of oxygen evolved in a unit of time or by titrating the excess of peroxid of hydrogen remaining after a given time.

Ori has also suggested another test based upon the fact that corn does not contain appreciable amounts of invertase, while most molds do. It is applied by putting 30 grams of meal into a flask with 90 cubic centimeters of 50 per cent aqueous solution of glycerin. After standing for twenty-four hours the extract is filtered off and twice its volume of 90 per cent alcohol added to it. The precipitate formed by the alcohol is collected upon a filter and dissolved in 45 c. c. of distilled water. Of this solution 2 c. c. are added to 50 c. c. of a 10 per cent cane-sugar solution and the mixture incubated for twenty-four hours at 50° C. It is then tested for reduction and the sugar titrated. Good meal should produce no reduction or only a minimal one. This test has not been used in this investigation.

THE DETERMINATION OF TOXICITY.

Much stress is laid in Italy upon the determination of toxicity. Schindler does not even mention it. It is performed as follows: A weighed quantity of meal is extracted at about body temperature with 90 per cent alcohol for twenty-four hours. It is then filtered and the alcoholic filtrate evaporated until the alcohol is removed. The residue is taken up in water at a temperature of 40° C., made up with warm water to a definite volume so that 0.5 c. c. corresponds to about 0.5 grams of the meal, and an amount equivalent to 0.5 grams of meal injected subcutaneously into a mouse. Larger quantities of liquid are often injected, but this seems open to objection in so small an animal. The mouse is chosen because it is supposed to be the most sensitive to the poison.[b] The symptoms are described as consisting of clonic spasms and localized contractures of the muscles, embarrassed respiration, gradual paralysis, collapse, death. Sometimes

[a] Ori, A., op. cit., p. 187.

[b] Gosio, B., and Ferrati, E., op. cit., p. 964.

opisthotonos ensues. On autopsy little is said to be noticeable except inflammation at the site of injection and hyperæmia of the cord.

A sample of corn which was toxic when injected in the dosage given above was never encountered in the present investigation. However, the procedure was varied from that of the Italians because of the following considerations: The extracts may be very acid. It is well known that herbivorous animals are very sensitive to acids which they are incapable of destroying in their metabolism. The symptoms of such an acid intoxication (acidosis) are, however, different from those described above. The behavior of mice toward acid intoxication is not known so far as a hasty search of the literature has shown. It is therefore conceivable that some of the toxic effects of the injection of corn extracts may merely have been acid effects. For these reasons the solutions injected were usually neutralized. Perhaps that is why toxic effects were not obtained. In this connection it is interesting to note that Gosio and Ferrati[a] distinctly state that alkali neutralizes the poison, and in another place that culture fluid of Penicillium cultures becomes less toxic as the culture grows older and its acidity diminishes.

TESTS FOR MICRO-ORGANISMS AND FOR A TENDENCY TO BECOME MOLDY.

The test for micro-organisms and the tendency to become moldy involves the quantitative determination of the number of organisms in the suspicious sample compared with a sound sample. The methods hitherto proposed for this purpose do not seem to be adequate. To devise improved ones and to determine the nature of the organisms present is beyond the limits of the present problem. This has been undertaken by Dr. Erwin F. Smith, of the Bureau of Plant Industry, and he will no doubt report in due time.

A number of other tests have been proposed by various authors, such as the application of Millon's test and the bromin water test, to corn extracts. They are based on the assumption that the toxic substances of spoiled corn are phenols. Neither seems to offer any special advantage.

These, then, are the chief methods hitherto used for determining the fitness of corn for food. Although the writers lay the most stress upon the determination of acidity, each of the other tests has its uses. Under ordinary circumstances the examination will probably have to be limited to the acidity determination, while the expert food chemist and bacteriologist will control his results by using a number of other methods and thus reach an estimate more nearly correct than any single method can give.

[a] Op. cit., p. 978.

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